Absorption and metabolism of avenanthramides and phenolic acids following intake of oat bran

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Abbreviations: ADME, absorption, distribution, metabolism and excretion; ND, not detected; sMRM, scheduled multiple reaction monitoring

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Abstract

Scope: Intake of wholegrain has been linked with reduced chronic disease mortality, with oat intake particularly notable for lowering blood cholesterol and glycemia. To better understand the complex nutrient profile of oats, we studied the absorption, distribution, metabolism and excretion (ADME) of phenolic acids and avenanthramides after ingestion of oats in humans.

Methods and results: After a 2d (poly)phenol-low diet, 7 healthy men provided urine 12h before, and 48h after, consuming 60g oat bran (7.8μmol avenanthramides, 139.2μmol phenolic acids) or a phenolic-low (traces of phenolics) control in a crossover design. Analysis by UPLC-MS/MS indicated that oat bran intake resulted in an elevation in urinary excretion of 33 phenolics relative to control, suggesting that they are oat bran-derived. Mean excretion levels were elevated between 0-2 and 4-8h, following oat bran intake, and amounted to a total of 34.8±7.2μmol total excretion (mean recovery: 23.6±4.9%), relative to control. The predominant metabolites included: vanillic acid, 4- and 3-hydroxyhippuric acids and sulfate-conjugates of benzoic and ferulic acids, which accounted for two thirds of total excretion.

Conclusion: Oat bran phenolics follow a relatively rapid ADME, with 33 metabolites excreted within 8h of intake, suggesting that bound phenolics are, in part, rapidly released by the microbiota.

1 INTRODUCTION

Increasing the daily intake of wholegrain cereals by 90g has been associated with reduction mortality from cardiovascular disease by 27%, total cancer by 15%, respiratory disease by 22%, diabetes by 51% and infectious diseases by 26%, as indicated by recent meta-analysis of 45 prospective studies [1]. Human intervention studies have to date largely focused on wholegrain oats (*Avena sativa*), with meta-analyses establishing that regular oat intake lowers blood cholesterol [2, 3] and improves insulin sensitivity and post-prandial glycemic control [4]. Although oats only account for 1% of world grain production, they, unlike more widely consumed grains, are almost exclusively consumed as wholegrains and therefore a rich dietary source of high quality proteins, minerals, vitamins, soluble β-glucan fiber and phenolic compounds (i.e. phenolic acids and avenanthramides), all of which are concentrated in the outer bran layers [5].

β-glucan is, at least in part, responsible for the health benefits of oats [2-4], while there is limited *in vitro* evidence that avenanthramides [6] and phenolic acids [7] also may also promote beneficial cardiovascular physiology. Phenolic acid intake in Europe is on average 605 mg/d, with the main dietary sources being coffee (75%), fruits (5.6%) and wholegrain products (5.5%) [8]. Wholegrain is the richest dietary source of ferulic acid, which has a mean intake of 38 mg/d in Europe [8], although a number of other phenolic acids and phenolic alkaloids, notably the avananthramides, are present in oats, either in the 'free form', as soluble conjugates, or as insoluble bound forms (including ester-linked to fiber) [9]. Understanding the absorption, distribution, metabolism and excretion (ADME) of oat phenolic compounds following the dietary intake of whole oats or oat bran is a key prerequisite for determining which phenolic metabolites may mediate the health benefits of oats.

Previous data using oat phenolic extracts or wheat have indicated that phenolics transfer to the circulation following intake [10, 11], whilst limited data exist for the ADME of phenolics and other bioactive components from whole oats or oat bran. Notably, the intake of 150 mg of avananthramides (highly concentrated oat extract), led to detection of avenanthramides 2c, 2f and 2p at nanomolar concentrations in plasma between 0.25 and 5h, peaking at 2h [11]. While this study did not examine metabolism, data from studies in animals suggest that avenathramide 2c is metabolized to avenanthramide 2f, dihydroavenanthramide 2f and 2c, four hydroxycinnamic acids, and 5-hydroxyanthranilic acid following oral intake [12]. Furthermore, intake of 94 g wholegrain wheat bread, containing 87 mg of ferulic acid or aleurone-enriched white bread led to the appearance of ferulic acid-sulfate, dihydroferulic-sulfate, hippuric acid and two hydroxyhippuric acids in plasma, with the former two reaching peak plasma concentrations of 84 nM and 9 nM at 1 and 7 h after intake, respectively [10], along with 12 other phenolic acid metabolites in 48h urine, suggesting that ferulic acid is subject to extensive metabolism and that some fiber-linked ferulic acid is released later during transit through the gastrointestinal track through action of the microbiota [10].

The aim of our study was to examine the ADME of avenanthramides and phenolic acids following intake of 60g oat bran. Specifically, the study focused on three objectives; 1) to establish peak urinary excretion intervals of phenolic acids and avenanthramides; 2) to determine the temporal nature of oat phenolic release from the bound state in the gastrointestinal tract; and 3), to identify and quantify the range of phenolic metabolites derived from the oat phenolics using comprehensive UPLC-MS/MS methods.

2 MATERIALS AND METHODS

2.1 Chemicals and reagents

Avenanthramide 2c, avenanthramide 2f, avenanthramide 2p, 2,4-dihydroxy benzoic acid, *p*-coumaric acid, caffeic acid, isoferulic acid, syringic acid, salicylic acid, o-coumaric acid, vanillic acid, syringaldehyde, ferulic acid, sinapic acid, 4-hydroxybenzaldehyde, 4-hydroxybenzoic acid, vanillin, protocatechuic acid, isovanillic acid, gallic acid, homovanillic acid, hippuric acid, dihydroferulic acid, dihydrocaffeic acid, 4-hydroxyphenylacetic acid and 2-hydroxyhippuric acid were obtained from Sigma-Aldrich (see Supporting Information Table 1 for IUPAC names). Dihydroferulic acid-4-O-glucuronide, isoferulic acid-3-O-sulfate, ferulic acid-4-O-glucuronide, dihydroxybenzoic acid-3-O-glucuronide and 5-hydroxyanthranilic acid were obtained from Toronto Research Chemicals Inc. 4-hydroxyhippuric acid and 3-hydroxyhippuric acid were purchased from Enamine (see Supporting Information Table 1 for IUPAC names). All solvents were HPLC grade and were obtained from Sigma-Aldrich or Fisher Scientific. While we acknowledge that avenanthramides are phenolic alkaloids, the terms phenolics is used throughout the paper to include both avenanthramides and phenolic acids.

2.2 Extraction and analysis of oat bran phenolics

Soluble and bound phenolic fractions were extracted from oats using an established method [13], with addition of hexane defatting steps adapted from [14] and preservation of phenolics during alkali hydrolysis using ascorbate and EDTA adapted from [15]. Phenolic acids and avenanthramides were separated using an Agilent 1100 series HPLC equipped with a Kinetex biphenyl column (100 Å 250x4.6 mm, 5µm; Phenomenex) with a SecurityGuard ultra biphenyl cartridge (Phenomenex). Sample injection volume was 20 µL, the flow rate 1 mL/min and mobile phases consisted of 0.1% formic acid (v/v) in water (solvent A) and 0.1% formic acid (v/v) in methanol (solvent B). The solvent gradient consisted of 5% B at 0 min, 25% B at 20

min, 26% B at 25 min, 35% B at 30 min, 36% B at 40 min, 70% B at 53 min, 95% B at 56 min, 95% B at 61 min, 5% B at 62 min, 5% B at 65 min. The absorbance was recorded at 254, 280 and 320nm and quantification was based on 12 point linear calibration curves (mean R^2 > 0.994) and as a ratio to the internal standard (i.e. 3,5-dichloro-4-hydroxybenzoic acid) to account for losses during extraction.

2.3 Study design

Seven healthy men aged 25-62 years were recruited from the local community. Exclusion criteria were as follows: recent (last 3 months) use of antibiotics, flu vaccination or dietary supplements. All participants gave written informed consent prior to study commencement and the study was performed at the Hugh Sinclair Unit of Human Nutrition, University of Reading (UK) between July 2014 and September 2014 and was approved by the University of Reading Research Ethics Committee (Reference number: 31/15), followed the principles of the Declaration of Helsinki and was registered on ClinicalTrials.gov under NCT02574039.

The study was designed as a non-blinded, randomized, controlled trial, where participants attended two experimental visits that were identical with the exception of the two study meals: 1) Test intervention consisting of 60 g oat bran porridge, 200 ml semi-skimmed milk and 100 ml water, microwaved (2min); or 2) a control consisting of two slices of white bread, 14g butter, golden syrup, one boiled egg and 200 ml semi-skimmed milk; given in random order. Participants arrived at the Nutrition Unit at 8am or 9am to consume the study meal within 10 min. Urine was collected at 11 specific intervals relative to study meal consumption: -12h to 0h (i.e. baseline), 0h to 2h, 2h to 4h, 4h to 6h, 6h to 8h, 8h to 12h, 12h to 24h, 24h to 28h, 28h to 32h, 32h to 36h and 36h to 48h (see Figure 1). Collected urine was kept on ice pack and immediately following extraction, aliquots were stored at -80°C with and without 5% formic

acid acidification. During the 48h urine collection period, participants consumed a low-phenolic meals provided to them (lunch: a white bread cheese sandwich and toffee yoghurt; dinner: macaroni cheese pasta, white bread roll and crème brulée; breakfast: white toast with butter and golden syrup, a boiled egg and glass of milk), whilst drinking water *ad libitum*. Prior to each experimental visit, participants followed a diet low in (poly)phenols for 48h (i.e. no fruits, vegetables, wholegrains, pulses, spices, herbs, nuts, seeds, chocolate, tea and coffee), and attended an overnight fast after having consumed a low-(poly)phenol dinner (a cheese pasta bake, white bread roll and crème brulée). Compliance to the dietary restrictions was assessed using food intake diaries and questionnaires. One volunteer was excluded from the final dataset, due to non-compliance to the dietary restrictions (i.e. volunteer reported intake of (poly)phenol-rich foods and baseline and post-control urine contained high levels of phenolics).

2.4 Solid phase extraction

Phenolic acids were extracted from urine using a validated method [16] with minor modifications. Briefly, 1 mL of urine was spiked with an internal standard (i.e. 3,5-dichloro-4-hydroxybenzoic acid) and subsequently extracted using solid phase extraction cartridges (Strata-X columns 500mg/6mL; Phenomenex). These were washed with 12 mL of 0.1/99.9 v/v hydrochloric acid/water, dried for 30 min under vacuum, soaked in 0.1/99.9 v/v hydrochloric acid/methanol for 10 min and eluted into glass vials with 7 mL 0.1/99.9 v/v hydrochloric acid/methanol. Samples were evaporated to complete dryness under speedvac at room temperature. The dried samples were resuspended in 250 μL of mobile phase (0.1/5/94.9, v/v/v, formic acid/methanol/water) by 30s vortexing, 15min ultrasound sonicating and 1h shaking. Samples were stored at -80°C until analysis. For phenolic acids and metabolites the method

has a mean ± SD extraction efficiency of 88.3 ± 17.8% [16], while we established extraction efficiencies of 102%, 97% and 57% for avenanthramide A, B and C, respectively.

2.5 UPLC-MS/MS analysis

The UPLC-electrospray ionisation-MS/MS system consisted of an Aquity UPLC Hclass (Waters) coupled to a Xevo TQ-S micro electrospray ionisation mass spectrometer (Waters) operated using MassLynx software (V4.1, Waters Inc, USA). Compound separation was achieved using an Aquity UPLC HSS T3 1.8µm column (2.1 x 100mm) attached to a Van guard pre-column of the same material and pore size, maintained at 45°C with a flow of 0.65 mL/min and a sample injection volume of 2 µL. The mobile phase consisted of 0.1/99.9 v/v formic acid/water (A) and 0.1/99.9 v/v formic acid/acetonitrile (B); and a mobile phase gradient consisting of: 1% B at 0 min, 1% B at 1 min, 30 % B at 10 min, 95 % B at 12 min, 95% B at 13 min, 1% B at 13.10 min, 1% B at 16 min. A scheduled multiple reaction monitoring (sMRM) method was developed by syringe infusion of 34 analytical standards (see section Chemicals and Reagents and Supporting Information Table 1) to determine sMRM transitions, optimal sMRM modes (i.e. negative or positive) and collision energies (Supporting Information Table 2).

Regarding phase II metabolites, while four authentic standards were used for the sMRM method (i.e. dihydroferulic acid-4-O-glucuronide, isoferulic acid-3-O-sulfate, ferulic acid-4-O-glucuronide and dihydroxybenzoic acid-3-O-glucuronide), further putative glucuronide or sulfate conjugated phenolic acid metabolites and feruloyl glycine were added to the sMRM method, even though analytical standards were not commercially available. For these, retention times and sMRM transitions were tentatively identified by injecting a pooled extract of urine (i.e. using urine collected after oat bran intake from all n=7 participants and during all 10 post-intake time intervals). sMRM transitions were taken from the literature [17-19] or derived from

the fragmentation pattern of the phenolic acid aglycones by adding the m/z of glucuronide (i.e. 176) or sulfate (i.e. 80) to the precursor ion and including the appropriate MS/MS fragment. Furthermore, 113 m/z, a common fragment of glucuronic acid, was added as an MS/MS fragment for glucuronide conjugates [19]. Collision energies were optimized to a limited extent by injecting the pooled urine extract three times at collision energies -11, -17 and -21 and the best one was selected for each sMRM transition. In the final sMRM, a total of 84 potential compounds were targeted and statistical comparison between the oat and control intervention was used to identify those which are oat-derived phenolics.

Quantification was established using the most intense sMRM transition and 11-14 point calibration curves of analytical standards (Supporting Information Table 2). Where pure standards were not available, quantification was conducted relative to standard curves of compounds with similar structures (e.g. the calibration curve of isoferulic acid-3-O-sulfate was used to quantify all tentatively identified sulfate metabolites). The limit of detection was established for each compound as the concentration of a peak with a signal to noise ratio of 3. A blank and quality control were run every 10 injections and the quality control indicated a between-run coefficient of variation of 10%.

Sample acidification using 5% formic acid did not significantly affect phenolic compound peak areas (established in n=3 volunteers; data not shown) and therefore non-acidified urine was used for the complete analysis.

2.6 Statistical analysis

A two-factor repeated-measurement linear mixed model was fitted to analyze hourly urinary excretion data. The model included participants nested within time as a random effect and baseline hourly urinary excretion, intervention, time and interaction as fixed effects. When the

model showed a significant interaction effect, *post-hoc* analysis with Tukey-Kramer adjustment was performed. Data are presented as means ± SEMs. P-values of <0.05 were considered statistically significant, and statistical analysis was performed by using R programming language version 3.1.2 (R Development Core Team, 2014).

3 RESULTS

3.1 Phenolic composition of the oat bran intervention

Twelve 12 phenolic compounds were detected in the oat bran used for the trial, with eight phenolic acids being present in both soluble and bound fractions, three avenanthramides only present in the soluble fraction and vanillin only in the bound fraction (Table 1). The intervention diet (60g oat bran) contained 28.6mg total phenolics (24% in the soluble fraction), with ferulic acid being the predominant phenolic acid (16.8mg) followed by *p*-coumaric acid (3.3mg) and the three avenanthramides collectively amounting to 2.5mg. Although the control meal and all other meals consumed during the 48h study periods were not analyzed, they consisted of white wheat bread and pasta and would therefore be expected to contain only low amounts of phenolic acids [20].

3.2 Identification of oat derived phenolic compounds in urine

A wide range of phenolic compounds were detected in urine at baseline and after the control meal intake (Supporting Information Table 3). However, the hourly excretion of 33 individual phenolic compounds was elevated following intake of oat bran compared to the control intervention (significant mixed model P-value of intervention and/or time*intervention; Supporting Information Table 3), suggesting that they are derived from the ingested oat bran.

These phenolics were identified as two avenanthramides and 31 phenolic acids (11 aglycones, four glycine conjugates, 10 sulfate conjugates and eight glucuronide conjugates; Supporting Information Table 2). Excretion of hippuric acid, the dominant phenolic acid in human urine, and ferulic acid aglycone were not statistically higher after the oat bran intervention relative to the control and were thus not identified as oat-derived metabolites (mixed model time*intervention interaction P-values: 0.36 and 0.06, respectively; Supporting Information Table 3).

3.3 Hourly urinary excretion of oat phenolic compounds

At baseline, the mean hourly excretion of the total excretion of 33 phenolic compounds prior to oat bran and control interventions was 2.5±0.6 μmol/h and 1.9±0.4 μmol/h, respectively (Figure 2). Following intervention, the total hourly phenolic excretion significantly increased at 0-2h (oat: 9.5±0.6 μmol/h, control: 1.9±0.4 μmol/h; post-hoc P-value <0.0001), returned to a similar level as control at 2-4h (oat: 4.8±0.9 μmol/h, control: 4.0±1.2μmol/h; post-hoc P-value P-value >0.05) and peaked again between the 4-6 h period (oat: 8.8±1.9μmol/h, control: 4.0±0.7μmol/h; post-hoc P-value < 0.05) and 6-8h (oat: 13.0±1.2μmol/h, control: 7.5±0.6μmol/h; post-hoc P-value < 0.01) before returning to a similar level to the control intervention from 8 to 48h (mixed model P-values: time < 0.0001, intervention <0.01 and time*intervention interaction <0.0001; Figure 2). Figure 3 shows the oat-bran induced increase in excretion of the individual 33 oat-derived phenolics proportional to the total excretion at 0-2h, 4-6h and 6-8h, relative to control. Vanillic acid, 4-hydroxyhippuric acid, 3-hydroxyhippuric acid, benzoic acid-sulfate and ferulic acid-sulfate were the predominant oat bran-derived phenolics excreted, accounting for more than two thirds of the total excretion (i.e. 20.3%, 16.1%, 14.3%, 9.8% and 7.3%, respectively). (Iso)ferulic acid-sulfate was predominant early during the early 0-2h peak, vanillic acid was almost exclusively excreted during the late 4-8h period, whilst excretion of hydroxyhippuric acids and benzoic acid-sulfate was biphasic, with

early and late peaks (Figure 3). Ferulic acid, *p*-coumaric acid and avenanthramides 2p and 2f (i.e. the most abundant phenolic compounds in oats) accounted only for small percentages of the total excreted phenolics (i.e. 0.18%, 0.03%, 0.02% and <0.01%, respectively; Figure 3), suggesting that they are subject to extensive metabolism.

The mean absolute urinary excretion of the 33 oat-derived phenolics from 0-8h was 72.3±0.8µmol after consumption of oat bran and 37.5±4.5µmol after the control intervention (Table 2). Hence, on average 23.6±4.9% of the ingested dose (i.e. 147µmol phenolics/60g oat bran; Table 1) was recovered in the 0-8h urine and there was large inter-individual variability in absorption that ranged from 4.8% to 41.6% (Table 2). The mean percentage recovery of metabolites was lowest for the avenanthramides (range: 0-0.3%), intermediate for hydroxycinnamic acids (6.3-10.6%) and highest for hydroxybenzoic acids (14.6-159.2%).

4 DISCUSSION

The present controlled trial examined ADME of phenolic metabolites following intake of oat bran over a 48h timeframe. Oat bran intake resulted in elevated urinary excretion of 33 phenolic compounds at 0-2h and 4-8h, with sulfate or glycine conjugated benzoic acids being the major metabolites together with ferulic acid sulfate, and the total amounts of phenolic compounds recovered in urine amounting to 23.6±4.9% of the ingested phenolic dose. These results suggest that a high proportion of oat phenolics are bioavailable as a wide range of metabolites with absorption occurring both in the small intestine and then in the large intestine within a few hours of consumption.

Analysis of the utilized oat bran detected nine phenolic acids amounting to 434 µg/g and three avenanthramides amounting to 41 µg/g (Table 1). These levels are comparable to four previous studies that analyzed wholegrain oats or oat bran, identifying up to 10 phenolic acids at levels ranging from 273 to 874 µg/g and three avenanthramides at levels ranging from 13 to 116 µg/g [9, 21-23]. The relatively wide range in the reported contents and compositions of phenolics can be explained by the different oat products analyzed (i.e. commercial or non-commercial varieties, bran or wholegrain, and hulled or de-hulled), and by the different extraction and analytical methods used for their analysis. Although other cereals may be richer sources of phenolic compounds (for example, Mattila et al. found 6.8-fold and 6.3-fold more total phenolic compounds in wheat and rye bran, respectively, than in oat bran [22]), oats are particularly interesting because of the relatively high proportions of phenolics in free and conjugated forms (24% in our oat bran and from 34 to 62% in non-commercial hulled wholegrain oats [9], whereas wheat has less than 18% [20]) and thus phenolic components from oats may have a higher bio-accessibility in comparison to other cereals.

We observed high background excretion of all 33 oat-derived phenolic compounds (Supporting Information Table 3; Figure 2), even though volunteers followed a 48h low-phenolic diet. A high background phenolic excretion has previously been described in the control groups of other studies of (poly)phenol bioavailability after following 2 [24] or 7d [25] low phenolic dietary restrictions. These phenolic compounds may originate from the relatively low amounts of phenolics consumed in white bread and pasta during the dietary restrictions [20] and from the metabolism of other dietary components such as aromatic amino acids [26]. Despite this high background excretion, our data indicate that intake of oat bran leads to urinary excretion of 33 phenolic acid compounds which peak early at 0-2h, and again later between 4-8h (Figure 2). The early appearance of metabolites suggests that the absorption of free phenolics from the oat bran occurs in the upper gastrointestinal tract with esterified conjugates being hydrolyzed

by esterase activity in the intestinal mucosa [27], followed by the transfer of free phenolics across the intestinal epithelium through passive diffusion or via transporters [28]. Although we hypothesized that phenolics in the bound fraction would appear late in the urine (> 12 h), due to a requirement for gut microbiota to cleave covalent linkages between phenolic and fiber, the second peak of excretion was complete by 8 h, suggesting that the release and absorption occurred more rapidly (Figure 2). This agrees with previous studies, showing that bound phenolics may reach the colon and undergo microbial fermentation within 4 hours of oat bran intake. Indeed, in fasted volunteers (which was true in our study) a mean mouth-to-cecum transit time can be as rapid as 2.3h [29] and *in vitro* fermentation of wheat bran with human microbiota shows that digestion by microbial esterase and xylanase starts within 2h resulting in most fiber-bound phenolics being released within 6h [30].

The urinary recovery of ingested phenolics was on average 23.6±4.9% in the first 8 h after the oat bran intake, relative to the control (Table 2). Previous non-controlled studies reported lower mean recoveries in 24h urine of 4 ± 1%, 8 ± 2% and 3 ± 1% following ingestion of phenolics in wholegrain wheat bread [10], aleurone-enriched wheat bread [10] or wheat bran cereals [18], respectively, but a higher mean recovery of 29 ± 4% following ingestion of phenolics in instant coffee [17]. This wide variation in phenolic bioavailability may partly be explained by differences in the bioaccessibility of phenolics within the food matrix. While phenolic acids in coffee are conjugated to quinic acids and are relatively water soluble [17], the oat bran used here had 24% soluble phenolic acids (Table 1) and wheat bran cereals only 9% [18]. However, such direct comparisons of recoveries between studies may be of limited value due to the non-controlled design of previous studies [10, 17, 18], and differences in the methods used to analyze phenolic acids in the urine and food samples including the number of metabolites targeted with the LC-MS/MS methods.

Possible pathways for the metabolism of 12 ingested oat phenolic compounds into the 33 excreted urinary compounds based on metabolic pathways previously described in the literature [17, 24, 31, 32] are provided in Supporting Information Figure 1. Notably, the major excreted compounds are downstream in the metabolic pathways (Figure 3 and Supporting Information Figure 1) and therefore products of extensive metabolism by endogenous and colonic microbial enzymes. Free forms of vanillic and isovanillic acids were the highest and 7th most highly excreted phenolics, respectively being mainly in the late peak between 4-8h after oat bran intake (Figure 3) and at levels totaling more than the total amount of vanillic acid that was ingested (mean ± SEM recovery: 159.2 ± 53.8%; Table 2). These data suggest that whilst only a limited amount of vanillic acid is absorbed directly in the small intestine, a larger proportion is absorbed following release by fermentation by the colonic microbiota with previous studies suggesting that it originated partly from the metabolism of avenanthramides and hydroxycinnamic acids [24, 31, 33, 34]. Vanillic acid may be formed by β-oxidation of ferulic acid in the liver, and isovanillic acid from methylation of caffeic acid to isoferulic acid followed by liver β-oxidation (Supporting Information Figure 1) [24, 31, 33, 34]. 4-, 3- and 2-hydroxyhippuric acids were also highly excreted, with their excretion following a biphasic pattern (early 0-2h and late 4-8h) after oat bran intake (Figure 3). While hydroxyhippuric acids are common flavonoid metabolites detected, for example, following intake of orange juice [35] and cocoa [36], results from this oat trial and a recent wheat trial [10] suggest that hydroxyhippuric acids are also important metabolites derived from wholegrain.

A study feeding 75-150mg avenanthramides in an oat extract detected nanomolar concentrations of circulating avenanthramides [11], while the present study detected only traces of the ingested 2.5mg avenanthramides in urine. This may suggest that avenanthramides are bioavailable when ingested at a relatively high dose and that avenanthramides are mostly metabolized particularly to hydroxycinnamic acids during their passage through the GI tract

and into the circulation (Table 2). Hydroxycinnamic acids, in turn, undergo reduction, methylation, sulfation or glucuronidation, and are also metabolized to smaller hydroxybenzoic acids (Table 2, Supporting Information Figure 1). Chlorogenic acid and caffeic acid aglycones in coffee have a similar metabolic fate to avenanthramides and hydroxycinnamic acids in cereals. A study in ileostomy patients showed that although 33% of chlorogenic acid and 95% of caffeic acid were absorbed in the small intestine, only traces of chlorogenic acid and 11% of caffeic acid of the ingested dose were excreted in the urine [37]. The ingested avenanthramide dose given in the present study was only 2.5mg and this low dose may have prevented us from identifying avenanthramide metabolites.

In conclusion, the present study showed that oat bran phenolics are bioavailable than previous studies reported [10, 17, 18] with 23.6±4.9% of the ingested dose being excreted in urine during 8h following intake in the form of 33 different phenolics or their metabolites. The data showed that benzoic acid derivatives, and in particular (iso)vanillic acid, and three isoforms of hydroxyhippuric acids, accounted for a high proportion of the excreted compounds, together with ferulic acid sulfate. Future work is required to establish the detailed pharmacokinetics and circulating concentrations of these oat-derived phenolic compounds and to determine their biological activities and contributions to the health benefits of a diet rich in oats.

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FIGURE LEGENDS

Figure 1: Study design overview

Figure 2: Urinary excretion rate of total 33 oat-derived phenolic compounds after intake of 60g oat bran or a control meal in healthy men (µmol/h). Data are reported as mean ± SEM and were analyzed by two-factor repeated measurement linear mixed model with time and intervention as the two factors [significant effect of intervention ($P = 0.004$), time ($P = 3*10^{-13}$), and time and intervention interaction ($P = 6*10^{-6}$)]. *Post-hoc* analysis with Tukey-Kramer adjustment was performed and P-values are indicated as follow: * $P < 0.05$; ** $P < 0.01$; **** $P < 0.0001$.

Figure 3: Proportions of individual oat derived phenolics relative to total excretion levels at 0-2h, 4-6h and 6-8h. Excretion level differences between the oat and control interventions were used to calculate percentages.

Author contributions

Study design (MYS, GC, GS, PRS, JPES), oat phenolic analysis (MYS, GS, AL, PRS), human study implementation (MYS, GS, AK, SNSA), solid phase extraction (MYS, GS, CD, AK, SNSA), mass spectrometry (MYS, GC, CD, VB), statistical analysis (MYS), data interpretation (MYS, GC, GS, PRS, JPES), manuscript preparation (MYS), manuscript approval (GC, VB, PRS, JPES).

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Disclaimer

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Table 1: Content and composition of phenolics in 60g oat bran (mg and µmol)[a)]

	Fraction		
	Conjugated and free	**Bound**	**% Conjugated and free**
Avenanthramides			
Avenanthramide 2c	0.8mg (2.5µmol)	ND	100%
Avenanthramide 2f	1.0mg (3.1µmol)	ND	100%
Avenanthramide 2p	0.7mg (2.2µmol)	ND	100%
Hydroxycinnamic acids			
Ferulic acid	2.0mg (10.1µmol)	14.8mg (76.4µmol)	12%
p-Coumaric acid	0.4mg (2.3µmol)	3.0mg (18.1µmol)	11%
Caffeic acid	0.1mg (0.4µmol)	1.3mg (7.0µmol)	5%
Sinapic acid	0.9mg (3.8µmol)	1.2mg (5.5µmol)	41%
Hydroxybenzoic acids			
4-Hydroxybenzoic acid	0.2mg (1.8µmol)	0.3mg (1.9µmol)	48%
Vanillic acid	0.4mg (2.4µmol)	0.4mg (2.2µmol)	53%
Syringic acid	0.4mg (2.1µmol)	0.3mg (1.5µmol)	57%
Benzaldehydes			
4-Hydroxybenzaldehyde	0.2mg (1.4µmol)	0.1mg (1.1µmol)	56%
Vanillin	ND	0.2mg (1.3µmol)	0%
Total	7.0mg (32.1µmol)	21.6mg (115.0µmol)	24%

[a)] 60g of oat bran porridge made with 200ml semi-skimmed milk and 100ml water. Heated by microwave. ND, not detected

Table 2: 0-8h absolute urinary excretion (µmol) of oat-derived phenolics and recovery of ingested dose (%)

	Mean±SEM urinary excretion 0-8h (µmol)			Recovery of ingested dose (%)		
Excreted phenolics	Control	Oat bran	Difference	Mean±SEM	Lowest/highest absorber	Oat parent compounds (Ingested dose)
Avenanthramides						
Avenanthramide 2p	0.006±0.001	0.013±0.003	0.007±0.002	0.3 ± 0.1%	0.0% /0.7%	Avenanthramide 2p (2.2µmol)
Avenanthramide 2f	0.000±0.000	0.001±0.000	0.001±0.000	0.0 ± 0.0%	0.0% /0.1%	Avenanthramide 2f (3.1µmol)
Hydroxycinnamic acids						
Ferulic acid metabolites[a)]	3.9±0.4	9.4±0.8	5.5±0.6	6.3 ± 0.7%	4.6% /8.5%	Ferulic acid (86.5µmol)
Caffeic acid-sulfate	0.4±0.2	1.2±0.4	0.8±0.5	10.7 ± 7%	-15.4% /34.9%	Caffeic acid (7.4µmol)
Sinapic acid-sulfate	0.5±0.1	1.5±0.2	1±0.2	10.6 ± 1.6%	4.9% /15%	Sinapic acid (9.3µmol)
Hydroxybenzoic acids						
(Iso)vanillic acid	6.2±2.5	13.5±3.1	7.3±2.5	159.2 ± 53.8%	-80.4% /308.3%	Vanillic acid (4.6µmol)
Benzoic acid-sulfate or -glucuronide	7.1±2	10.3±2.7	3.2±1	85.2 ± 26%	8.1% /176.1%	4-hydroxybenzoic acid (3.7µmol)
Syringic acid-sulfate	0.4±0.1	0.9±0.2	0.5±0.1	14.6 ± 4%	1.5% /30.5%	Syringic acid (3.6µmol)
Total 33 phenolics	37.5±4.5	72.3±8.0	34.8±7.2	23.6 ± 4.9%	4.8% /41.6%	Total 12 phenolics (147.1µmol)

[a)] Sum of ferulic, isoferulic, dihydroferulic and dihydroisoferulic acids: aglycones, glucuronides or sulfates

Figure 1

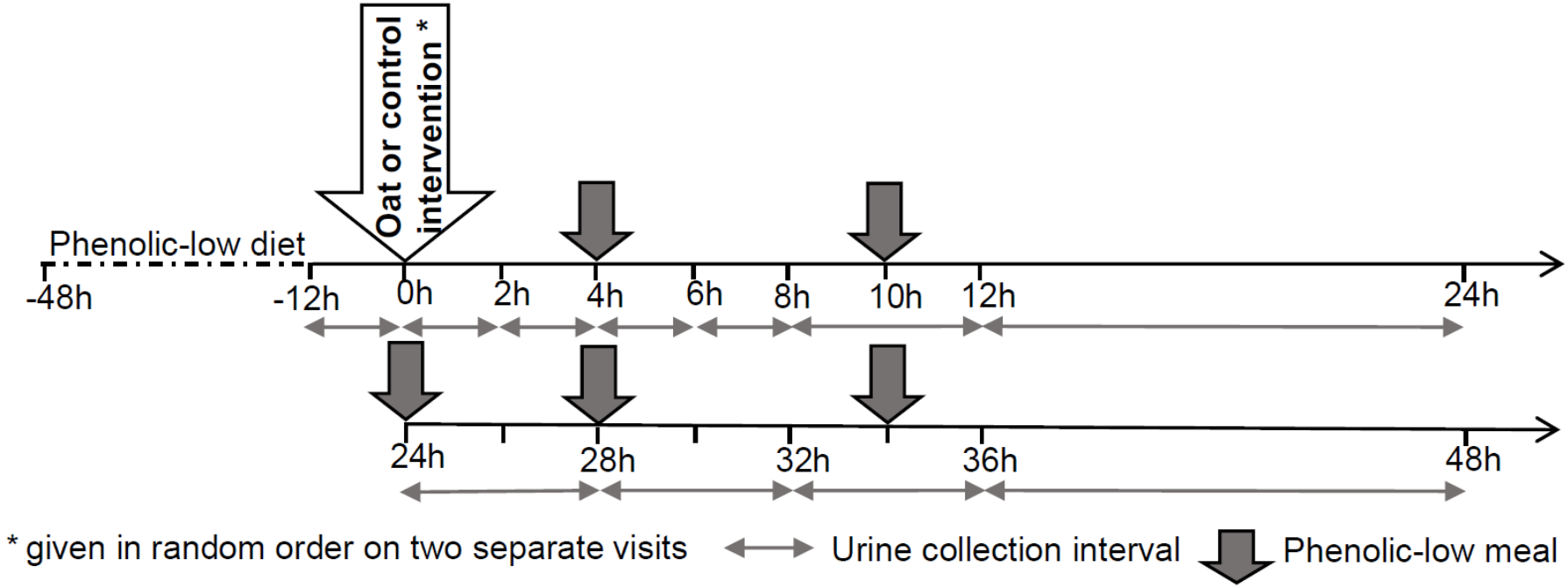

Oat or control intervention *
Phenolic-low diet
-48h
-12h
0h
2h
4h
6h
8h
10h
12h
24h
24h
28h
32h
36h
48h
* given in random order on two separate visits
Urine collection interval
Phenolic-low meal

Figure 2

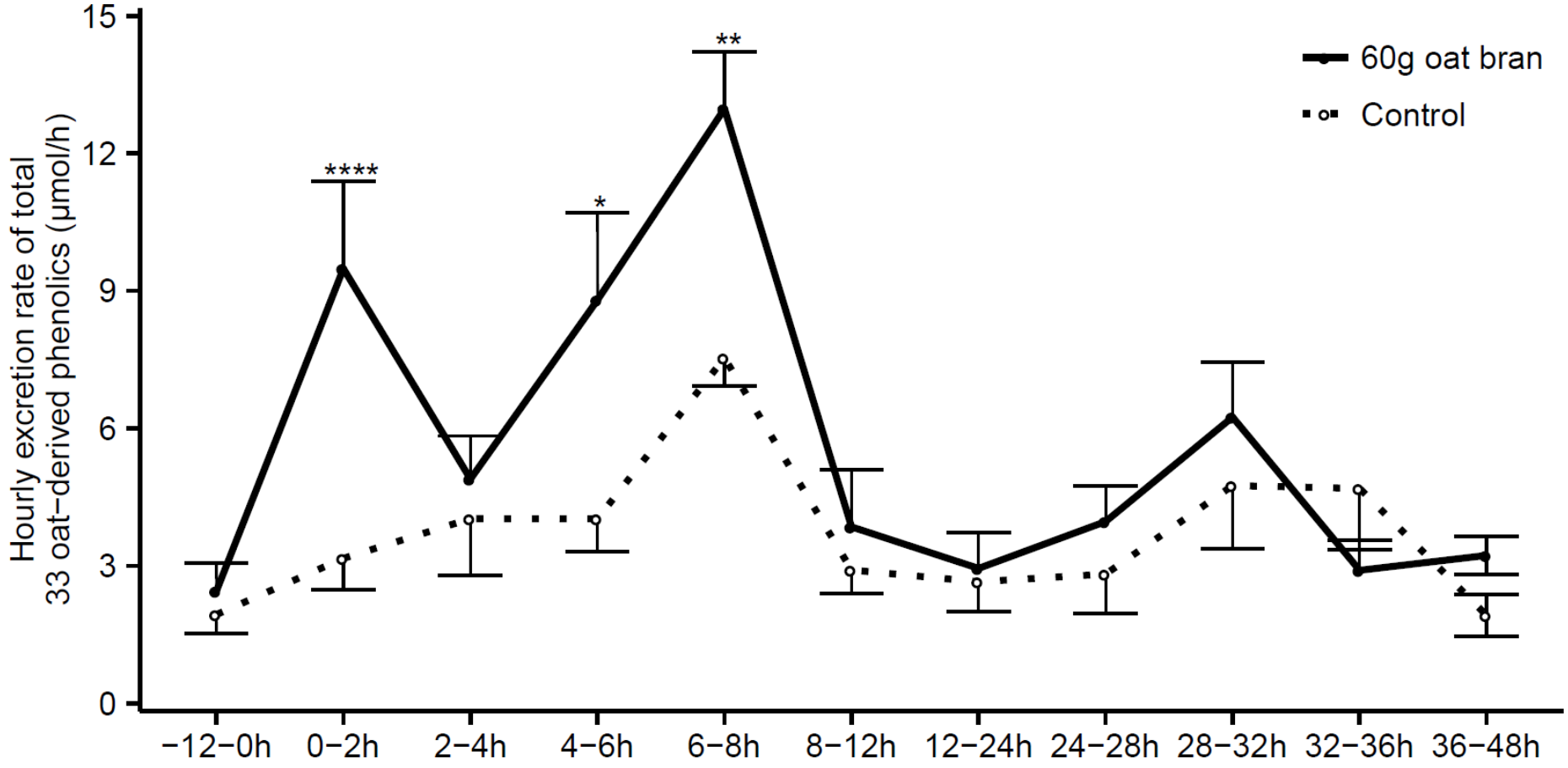
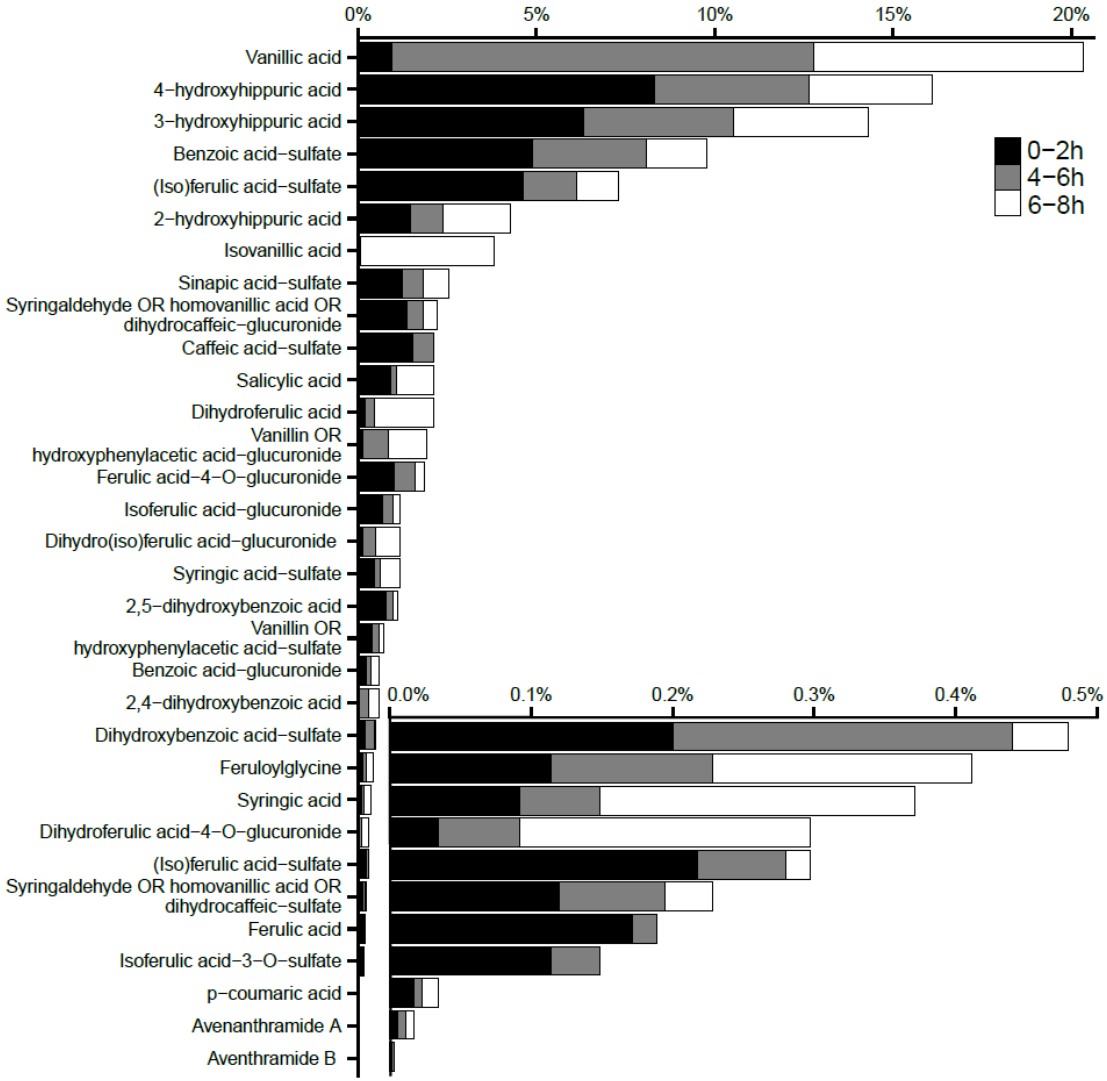

Hourly excretion rate of total
33 oat-derived phenolics (μmol/h)
15
12
9
6
3
0
****
*
**
60g oat bran
Control
−12−0h
0−2h
2−4h
4−6h
6−8h
8−12h
12−24h
24−28h
28−32h
32−36h
36−48h

Figure 3.


0%
5%
10%
15%
20%
Vanillic acid
4−hydroxyhippuric acid
3−hydroxyhippuric acid
Benzoic acid−sulfate
(Iso)ferulic acid−sulfate
2−hydroxyhippuric acid
Isovanillic acid
Sinapic acid−sulfate
Syringaldehyde OR homovanillic acid OR dihydrocaffeic−glucuronide
Caffeic acid−sulfate
Salicylic acid
Dihydroferulic acid
Vanillin OR hydroxyphenylacetic acid−glucuronide
Ferulic acid−4−O−glucuronide
Isoferulic acid−glucuronide
Dihydro(iso)ferulic acid−glucuronide
Syringic acid−sulfate
2,5−dihydroxybenzoic acid
Vanillin OR hydroxyphenylacetic acid−sulfate
Benzoic acid−glucuronide
2,4−dihydroxybenzoic acid
0−2h
4−6h
6−8h
0.0%
0.1%
0.2%
0.3%
0.4%
0.5%
Dihydroxybenzoic acid−sulfate
Feruloylglycine
Syringic acid
Dihydroferulic acid−4−O−glucuronide
(Iso)ferulic acid−sulfate
Syringaldehyde OR homovanillic acid OR dihydrocaffeic−sulfate
Ferulic acid
Isoferulic acid−3−O−sulfate
p−coumaric acid
Avenanthramide A
Aventhramide B

Supporting Information Table 1 – Common and IUPAC names of phenolic compounds

Common Name	IUPAC Name
List of available analytical standards	
2,4-dihydroxy benzoic acid	2,4-dihydroxybenzoic acid
2,5-dihydroxy benzoic acid	2,5-dihydroxy benzoic acid
2-hydroxyhippuric acid	2-[(2-hydroxybenzoyl)amino]acetic acid
3-hydroxyhippuric acid	2-[(3-hydroxybenzoyl)amino]acetic acid
4-hydroxybenzaldehyde	4-hydroxybenzaldehyde
4-hydroxybenzoic acid	4-hydroxybenzoic acid
4-hydroxyhippuric acid	2-[(4-hydroxybenzoyl)amino]acetic acid
4-hydroxyphenylacetic acid	2-(4-hydroxyphenyl)acetic acid
5-hydroxyanthranilic acid	2-amino-5-hydroxybenzoic acid
Avenanthramide 2c	2-[[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]amino]-5-hydroxybenzoic acid
Avenanthramide 2f	5-hydroxy-2-[[(E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoyl]amino]benzoic acid
Avenanthramide 2p	5-hydroxy-2-[[(E)-3-(4-hydroxyphenyl)prop-2-enoyl]amino]benzoic acid
Caffeic acid	(E)-3-(3,4-dihydroxyphenyl)prop-2-enoic acid
Dihydrocaffeic acid	3-(3,4-dihydroxyphenyl)propanoic acid
Dihydroferulic acid	3-(4-hydroxy-3-methoxyphenyl)propanoic acid
Dihydroferulic acid-4-O-glucuronide	(2S,3S,4S,5R,6S)-6-[4-(2-carboxyethyl)-2-methoxyphenoxy]-3,4,5-trihydroxyoxane-2-carboxylic acid
Dihydroxybenzoic acid-3-O-glucuronide	(2S,3S,4S,5R,6S)-6-(5-carboxy-2-hydroxyphenoxy)-3,4,5-trihydroxyoxane-2-carboxylic acid
Ferulic acid	(E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid
Ferulic acid-4-O-glucuronide	(2S,3S,4S,5R,6S)-6-[4-[(E)-2-carboxyethenyl]-2-methoxyphenoxy]-3,4,5-trihydroxyoxane-2-carboxylic acid
Gallic acid	3,4,5-trihydroxybenzoic acid
Hippuric acid	2-benzamidoacetic acid
Homovanillic acid	2-(4-hydroxy-3-methoxyphenyl)acetic acid
Isoferulic acid	(E)-3-(3-hydroxy-4-methoxyphenyl)prop-2-enoic acid
Isoferulic acid-3-O-sulfate	(E)-3-[3-sulfooxy-4-(methoxy)phenyl]prop-2-enoic acid
Isovanillic acid	3-hydroxy-4-methoxybenzoic acid
o-coumaric acid	(E)-3-(2-hydroxyphenyl)prop-2-enoic acid
p-coumaric acid	(E)-3-(4-hydroxyphenyl)prop-2-enoic acid
Protocatechuic acid	3,4-dihydroxybenzoic acid
Salicylic acid	2-hydroxybenzoic acid
Sinapic acid	(E)-3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enoic acid
Syringaldehyde	4-hydroxy-3,5-dimethoxybenzaldehyde
Syringic acid	4-hydroxy-3,5-dimethoxybenzoic acid
Vanillic acid	4-hydroxy-3-methoxybenzoic acid
Vanillin	4-hydroxy-3-methoxybenzaldehyde
Tentatively identified oat-derived phenolics	

3,4-dihydroxyhydrocinamic acid-glucuronide	(2S,3S,4R,5R,6S)-3-(5-(2-carboxyethyl)-2-hydroxyphenoxy)-4,5,6-trihydroxytetrahydro-2H-pyran-2-carboxylic acid
4-hydroxyphenylacetic acid-glucuronide	(2S,4R,5R,6S)-3-(4-(carboxymethyl)phenoxy)-4,5,6-trihydroxytetrahydro-2H-pyran-2-carboxylic acid
Benzoic acid-glucuronide	(2S,3S,4R,5R,6S)-3-(4-carboxyphenoxy)-4,5,6-trihydroxytetrahydro-2H-pyran-2-carboxylic acid
Benzoic acid-sulfate	4-carboxyphenyl sulfate
Caffeic acid-sulfate	(E)-4-(2-carboxyvinyl)-2-hydroxyphenyl sulfate
Dihydroferulic acid-glucuronide	(2S,3S,4R,5R,6S)-3-(4-(2-carboxyethyl)-2-methoxyphenoxy)-4,5,6-trihydroxytetrahydro-2H-pyran-2-carboxylic acid
Dihydroxybenzoic acid-sulfate	4-carboxy-2-hydroxyphenyl sulfate
Feruloylglycine	2-[[(E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoyl]amino]acetic acid
Homovanillic acid-glucuronide	(2S,3S,4S,5R)-6-(4-(carboxymethyl)-2-methoxyphenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid
Hydroxyhippuric acid-glucuronide	(2S,3S,4R,5R,6S)-3-(4-((carboxymethyl)carbamoyl)phenoxy)-4,5,6-trihydroxytetrahydro-2H-pyran-2-carboxylic acid
Hydroxyphenylacetic acid-sulfate	4-(carboxymethyl)phenyl sulfate
(Iso)ferulic acid-glucuronide	(2S,3S,4R,5R,6S)-3-(4-((E)-2-carboxyvinyl)-2-methoxyphenoxy)-4,5,6-trihydroxytetrahydro-2H-pyran-2-carboxylic acid
(Iso)ferulic acid-sulfate	4-(2-carboxyethyl)-2-methoxyphenyl sulfate
Sinapic acid-sulfate	(E)-4-(2-carboxyvinyl)-2,6-dimethoxyphenyl sulfate
Syringaldehyde-glucuronide	(2S,3S,4R,5R,6S)-3-(4-formyl-2,6-dimethoxyphenoxy)-4,5,6-trihydroxytetrahydro-2H-pyran-2-carboxylic acid
Syringaldehyde-sulfate	4-formyl-2-hydroxy-6-methoxyphenyl sulfate
Syringic acid-sulfate	4-carboxy-2,6-dimethoxyphenyl sulfate
Vanillin-glucuronide	(2S,3S,4R,5R,6S)-3-(4-formyl-2-methoxyphenoxy)-4,5,6-trihydroxytetrahydro-2H-pyran-2-carboxylic acid
Vanillin-sulfate	4-formyl-2-methoxyphenyl sulfate

Supporting Information Table 2 – sMRM transitions, parameters and detection limits of 33 identified oat phenolics and hippuric acid

Metabolite	RT	MW	sMRM ion transitions (m/z)	Collision energy (V)	LOD (nM)	R^2
Aglycones						
2,5-dihydroxybenzoic acid	4.8	154.1	153 / 109	-11	4.7	0.999
2,4-dihydroxybenzoic acid	5.5	154.1	153 / 109	-11	7.3	0.999
Vanillic acid	5.6	168.2	169 / 93	12	9.8	0.994
Isovanillic acid	6.0	168.2	169 / 93	12	7.3	0.999
Syringic acid	6.5	198.2	197 / 182	-13	14.9	0.999
Dihydroferulic acid	7.1	196.2	195 / 136	-15	21.5	1.000
p-Coumaric acid	7.3	164.2	163 / 119	-13	4.2	0.997
Ferulic acid	7.7	194.2	193 / 178	-10	8.2	1.000
Salicylic acid	7.8	138.1	137 / 93	-23	4.2	0.999
Avenanthramide 2p	9.9	299.3	298 / 254	-17	0.8	0.994
Avenanthramide 2f	10.1	329	328 / 268	-24	0.6	0.990
Glycines						
4-hydroxyhippuric acid	4.4	195.2	194 / 100	-11	4.3	0.997
3-hydroxyhippuric acid	4.7	195.2	194 / 100	-11	24.5	0.999
Hippuric acid[a)]	5.7	179.2	178 / 134	-11	24.1	0.995
2-hydroxyhippuric acid	7.0	195.2	194 / 100	-11	2.9	0.998
Feruloylglycine	6.4	251.2	250 / 100*, 206, 191, 177, 149	-11	2.2	no standard
Glucuronides						
Syringaldehyde OR Homovanillic acid OR 3,4-dihydroxyhydrocinamic acid-glucuronide[b,c)]	4.2	358.2	357 / 113* , 59 , 137 , 175 , 181	-17, -23, -23, -11, -17	20.7	no standard
Hydroxyhippuric acid-glucuronide[b)]	4.8	371.2	370 / 194* , 93 , 100 , 150 , 175 , 113	-11, -23, -11, -17	21.4	no standard
Benzoic acid-glucuronide[b)]	5.1	314	313 / 113* , 175 , 137 , 93	-17, -17, -17, -23	15.7	no standard
Ferulic Acid-4-O-Glucuronide	5.3	370.3	369 / 193* , 178 , 113 , 175	-19, -23, -19, -10	21.4	0.997
Vanillin OR 4-hydroxyphenylacetic acid-glucuronide[b,c)]	5.3	328.1	327 / 113* , 107 , 175 , 151	-17, -23, -11, -11	35.0	no standard
Dihydroferulic acid-4-O-glucuronide	5.8	372.3	371 / 113* , 195 , 85 , 175	-17, -20, -23, -17	11.7	0.990
Isoferulic acid-glucuronide[b)]	6.1	370	369 / 193* , 178 , 113 , 175	-19, -23, -19, -10	11.2	no standard
Dihydroferulic acid-glucuronide[b)]	6.2	372.3	371 / 113* , 195 , 85 , 175	-17, -20, -23, -17	8.0	no standard
Sulfates						
Dihydroxybenzoic acid-sulfate[b)]	3.0	234	233 / 109* , 189, 153, 97	-23, -11, -23, -23	3.6	no standard
Benzoic acid-sulfate[b)]	3.3	218	217 / 137*, 173	-11, -11	5.7	no standard
Syringaldehyde-sulfate[b)]	4.1	262.2	261 / 166* , 123 , 181	-23, -23, -11	3.1	no standard
Syringic acid-sulfate[b)]	4.1	278.2	277 / 197* , 182 , 153 , 123	-11, -23, -23, -23	2.6	no standard
Caffeic acid-sulfate[b)]	5.4	260.2	259 / 179* , 135 , 107	-17, -23, -23	7.7	no standard
Sinapic acid-sulfate[b)]	5.6	304.2	303 / 223* , 208 , 164 , 120	-11, -23, -23, -23	1.6	no standard
(Iso)ferulic acid-sulfate[b)]	5.7	274.2	273 / 178* , 192 , 134	-20, -16, -25	1.6	no standard
(Iso)ferulic acid-sulfate[b)]	5.9	274.2	273 / 178* , 192 , 134	-20, -16, -25	1.5	no standard
Isoferulic acid 3-O-sulfate	6.1	274.2	273 / 178* , 192 , 134	-20, -16, -25	1.4	1.000
Vanillin or hydroxyphenylacetic acid-sulfate[b)]	6.4	232.1	231.1 / 151*, 136, 92	-17, -23, -23	2.7	no standard

*sMRM transition used for quantification; [a)]Hippuric acid, a common urinary excretion compound, was not identified as an oat-derived metabolite; [b)] site of conjugation could not be ascertained as identification was based on known transitions where pure standards for isomers were not available or separation of isomers was chromatographically not possible; [c)]tentatively identified compound which could have different isomeric configuration. Abbreviations: LOD, limit of detection (Signal/Noise = 3); MW, molecular weight; RT, Retention time; sMRM, scheduled multiple reaction monitoring; R^2, linear regression coefficient of standard curve; no standard, no analytical standard was available to make standard curve

Supporting Information Table 3 – Urinary excretion rate per hour of oat-derived phenolic compounds after intake of 60g oat bran or a control meal in six healthy men (nmol/h)

													Model P value		
Metabolite	**Meal**	**-12-0h**	**0-2h**	**2-4h**	**4-6h**	**6-8h**	**8-12h**	**12-24h**	**24-28h**	**28-32h**	**32-36h**	**36-48h**	**Inter-vention**	**Time**	**Inter-action**
							µmol/h								
Avenanthramide A	Oat	1±0	2±1**	1±0	2±0*	2±0	1±0	1±0	2±0	1±0	1±0	1±0	***	**	**
	CON	1±0	1±0	1±0	1±0	1±0	1±0	1±0	1±0	1±0	1±0	1±0			
Aventhramide B	Oat	0±0	0.3±0	0.3±0.1	0.2±0**	0.1	0	ND	ND	ND	0.1	ND	**	**	*
	CON	0±0	0	ND	ND	ND	ND	ND	ND	ND	ND	0.1			
Ferulic acid	Oat	9±4	51±16	25±7	21±3	41±9	15±5	15±8	15±3	20±8	10±4	13±3	0.37	****	0.06
	CON	7±1	21±4	13±4	18±6	48±23	25±13	24±13	14±3	26±5	23±9	8±3			
p-coumaric acid	Oat	1±0	6±2*	3±0	3±1	5±0	2±1	1±0	2±1	2±0	2±1	1±0	*	***	*
	CON	2±1	3±1	2±1	2±0	3±0	3±2	2±1	1±0	2±0	2±1	1±0			
Dihydroferulic acid	Oat	29±15	90±42	25±9	60±17	378±135****	109±68	35±17	23±5	78±22	50±19	23±6	0.07	****	***
	CON	11±4	56±28	26±8	17±10	87±34	38±24	32±10	24±5	101±66	84±55	18±5			
Isovanillic acid	Oat	2±2	13±11	23±12	69±18	744±470****	54±51	14±12	9±4	157±115	54±44	16±10	0.05	**	*
	CON	4±4	9±9	9±7	62±14	87±39	3±1	1±0	1±0	59±17	4±1	1±0			
Syringic acid	Oat	5±2	29±5	12±3	18±5	55±19****	17±9	6±2	12±2	14±3	7±3	6±1	*	****	***
	CON	2±1	13±3	12±3	8±2	16±3	7±2	8±1	19±7	14±3	13±4	4±1			
Salicylic acid	Oat	67±59	171±64	143±54	64±22	222±120	31±16	17±5	95±66	103±36	109±82	30±14	**	0.05	0.21
	CON	6±2	14±6	39±17	32±12	43±7	24±11	11±5	20±9	22±8	24±8	9±3			
2,4-dihydroxybenzoic acid	Oat	4±1	15±1	10±3	54±26*	62±18***	13±5	19±13	10±2	36±19	8±3	8±2	**	**	***
	CON	4±1	11±3	14±5	6±2	11±4	6±2	5±2	18±8	5±2	6±2	4±2			
2,5-dihydroxybenzoic acid	Oat	51±17	209±58***	65±15	87±15	97±13	41±11	27±9	32±10	23±6	17±6	27±11	0.29	****	*
	CON	47±9	73±24	70±24	53±20	73±15	34±11	29±16	40±24	27±16	43±31	20±8			
Vanillic acid	Oat	29±14	175±36	67±14	3352±743**	2918±624*	201±98	48±21	39±12	1600±600	168±71	56±21	*	****	*
	CON	10±7	9±5	41±14	1281±624	1594±702	45±33	13±9	23±7	1162±513	128±37	25±17			
Hippuric acid	Oat	2772±703	9454±2193	7027±2028	7653±2036	12773±1494	5060±2449	4158±1001	5857±1432	6817±1545	5006±1627	4930±683	0.12	****	0.36
	CON	2403±339	5052±1396	7131±1944	5439±1199	10688±1668	4905±592	3905±1209	3593±1437	6311±2403	6525±1869	2696±853			
2-hydroxyhippuric acid	Oat	111±35	371±173*	133±35	245±104	525±82***	182±74	137±28	198±107	133±37	81±21	117±27	**	****	**
	CON	101±21	113±29	121±32	84±17	195±20	101±21	58±23	54±20	99±41	158±55	58±20			
3-hydroxyhippuric acid	Oat	443±148	1724±557**	958±278	1142±409	1643±302	332±126	452±98	599±209	650±329	288±75	557±98	*	****	**
	CON	395±89	617±203	729±226	405±140	981±271	364±97	451±259	344±223	248±103	820±325	355±126			
4-hydroxyhippuric acid	Oat	553±133	2262±551***	1289±289	1566±457	2479±79	939±348	748±187	1108±274	1302±298	885±228	899±105	0.11	****	**
	CON	430±71	809±232	1133±342	807±195	1875±246	820±105	628±221	650±247	1020±334	1314±307	528±167			
Feruloylglycine	Oat	11±5	32±12	11±2	28±8	56±18**	18±9	11±3	8±2	15±5	11±4	12±3	0.11	****	**
	CON	9±3	12±3	9±3	8±1	24±6	14±3	14±4	9±3	19±5	28±10	7±2			
Caffeic acid-sulfate	Oat	68±31	298±127***	90±38	128±41	87±26	23±10	18±7	11±4	24±12	14±5	12±4	0.64	0.06	***
	CON	65±27	28±8	13±4	30±12	137±98	110±84	47±19	18±6	76±51	103±62	22±9			
Syringaldehyde OR homovanillic acid OR	Oat	13±3	38±9*	21±6	33±9	46±10	21±8	13±4	19±2	32±6	19±4	14±2	0.16	****	*

dihydroxyhydrocinamic acid sulfate															
	CON	12±3	17±3	29±8	20±3	40±11	15±2	13±2	18±4	32±9	27±9	9±1			
(Iso)ferulic acid-sulfate	Oat	78±25	879±142****	560±104	474±57	661±119	245±82	101±36	170±39	359±76	219±71	118±29	****	****	****
	CON	48±14	71±20	282±63	210±51	454±87	135±31	79±22	121±54	235±74	131±58	70±25			
(Iso)ferulic acid-sulfate	Oat	6±1	43±7****	29±8**	20±4	28±6	12±5	7±2	8±1	16±3	10±3	7±1	**	****	****
	CON	5±2	5±1	13±3	9±1	25±4	9±2	6±1	7±2	15±4	14±4	5±1			
Isoferulic acid-3-O-sulfate	Oat	2±0	23±3****	16±5	12±1	7±1	11±6	3±1	1±0	4±1	4±2	3±2	0.12	***	****
	CON	1±0	3±1	9±4	6±2	12±7	4±2	2±1	6±3	7±4	6±3	3±1			
Syringic acid-sulfate	Oat	19±8	99±20***	54±9	51±10	165±54****	76±29	27±10	41±8	47±9	32±7	23±4	**	****	****
	CON	15±6	22±9	58±15	20±4	68±16	26±9	22±8	35±12	51±16	52±16	13±3			
Sinapic acid-sulfate	Oat	40±15	249±55****	108±14	144±21	252±75**	66±23	51±17	55±13	103±20	49±11	34±6	**	****	****
	CON	28±15	31±13	59±12	41±8	130±17	57±25	45±15	40±11	101±33	78±27	32±10			
Dihydroxybenzoic acid-sulfate	Oat	24±6	53±13*	27±7	51±12***	35±7	16±4	18±6	16±1	18±4	18±5	25±4	0.22	0.07	****
	CON	16±5	18±2	14±5	9±2	28±6	23±8	26±8	11±2	18±6	37±18	17±6			
Benzoic acid-sulfate	Oat	596±209	1697±494	720±219	1230±451	1283±274	1062±359	863±351	1034±274	1125±368	580±236	852±270	0.49	*	*
	CON	506±194	842±251	935±446	668±213	988±207	707±162	819±149	1073±295	1146±444	1066±464	461±143			
Vanillin or hydroxyphenylacetic acid-sulfate	Oat	13±9	81±50***	19±11	40±23	29±15	12±7	12±5	20±11	13±6	9±3	9±3	0.27	*	*
	CON	4±1	11±7	6±3	6±3	9±4	6±3	7±3	9±5	7±3	6±2	7±4			
Ferulic Acid-4-O-Glucuronide	Oat	49±20	221±69****	171±45	170±27	214±33	62±23	61±22	57±14	128±30	67±26	52±11	****	****	****
	CON	45±12	44±10	85±20	67±12	171±21	75±19	58±9	45±16	123±25	101±40	38±8			
Isoferulic acid-glucuronide	Oat	22±6	142±34****	94±29	78±14	104±23	36±32	23±5	29±7	58±11	43±15	25±4	*	****	**
	CON	17±4	21±5	39±9	29±5	67±8	33±6	20±3	20±6	57±7	41±9	15±4			
Dihydroferulic acid-4-O-glucuronide	Oat	3±1	11±4	9±2	16±5	49±22****	21±10	7±4	7±3	9±2	10±3	8±3	**	***	**
	CON	3±1	5±2	5±1	6±2	13±5	7±4	5±2	5±1	8±5	13±8	7±2			
Dihydro(iso)ferulic acid-glucuronide	Oat	31±10	54±17	18±5	73±20	189±64****	69±30	42±12	29±8	45±10	53±13	36±9	0.05	****	**
	CON	27±8	29±6	14±3	14±5	68±25	46±15	35±7	21±4	39±18	92±44	27±12			
Benzoic acid-glucuornide	Oat	23±8	83±26	28±5	45±15	78±15**	36±11	34±7	37±14	29±6	16±4	23±5	***	****	*
	CON	27±8	43±10	31±7	22±4	36±5	19±3	23±5	24±9	21±5	30±12	19±9			
Syringaldehyde OR Homovanillic acid OR 3,4-dihydroxyhydrocinamic acid-glucuronide	Oat	128±30	440±148*	172±45	176±53	273±67	155±31	139±37	267±60	142±40	179±47	203±29	0.87	***	**
	CON	68±27	201±66	208±61	95±15	203±40	141±37	177±34	179±37	109±34	211±46	131±32			
Vanillin OR 4-hydroxyphenylacetic acid-glucuronide	Oat	12±6	39±16	32±14	134±36****	212±27****	60±22	26±8	19±3	19±5	16±3	14±3	****	****	****
	CON	10±6	14±6	12±8	9±3	28±7	15±2	17±2	12±2	11±4	20±4	11±4			
Hippuric acid-glucuronide	Oat	2±1	7±1	7±0	17±4	25±4****	6±3	3±1	3±0	3±1	2±1	2±1	**	0.08	**
	CON	1±0	4±1	2±1	2±0	4±0	3±1	2±0	2±1	4±1	2±0	2±0			
Hydroxyhippuric acid-glucuronide	Oat	5224±1199	18942±3806*	11925±2840	16455±3736	25747±2324	8914±3675	7106±1750	9827±1978	13065±2602	7912±2179	8162±1049	*	****	*
	CON	4343±586	8219±1978	11163±3008	9471±1769	18227±2049	6999±998	6562±1753	6417±2263	11065±3732	11217±3195	4619±1259			

Data are reported as mean ± SEM and were analysed by two-factor repeated measurement linear mixed model with time and treatment as the two factors. When the interaction effect was significant, post-hoc analyses with Tukey-Kramer adjustment were performed. If excretion was ND, the concentration was replaced with the limit of detection for statistical analysis. Model P values in the three far right columns and post-hoc P values next to the excretion rates are indicated as follow: * P < 0.05; ** P <0.01; *** P<0.001; **** P<0.0001. ND; not detected; CON control.

Supporting Information Figure 1 – Possible metabolic pathways on how the 12 ingested oat phenolic compounds are metabolized into 33 excreted urinary compounds. The 5 top metabolites are highlighted with a blue background. COMT, catechol-O-methyl transferase, β-Ox, β-oxidase, GT, glucuronic acid transferase; ST, sulfate transferase; CoA, co-enzyme A